Effects of Restraint Stress and Allopregnanolone Inhibition on Amphetamine Locomotor Sensitivity

INTRODUCTION

The chronic, recurring nature of addiction remains a worldwide problem. Even after apparently successful clinical treatment and long-term abstinence, individuals may still relapse many months or years later. Although many individual differences exist among substance abusers, relapse tends to occur during periods of high stress (Sinha et al., 2006). Behavioral training and therapy can help cope during these high stress times, but pharmacological interventions have not been shown to be effective (Ross & Peselow, 2009). Although some therapeutic options decrease relapse rates, more effective treatments for relapse need further research.

The effect of stress on use and relapse to drugs of abuse likely stems from coupled stress and reward circuits in the brain. Stress leads to increased release of stress-related hormones including 3α, 5α tetrahydroprogesterone or, allopregnanolone (Purdy et al., 1991). Allopregnanolone is a neurosteroid tied to several brain circuits involved with stress and reward. Elevated levels of this neurosteroid occur throughout the mammalian brain and periphery after cocaine administration, and rats show enhanced dopamine release in the nucleus accumbens after an injection of finasteride, which inhibits the 5α-reductase involved in allopregnanolone synthesis (Dazzi et al., 2002). Finally, acutely stressed rats exhibit increased dopamine release in the prefrontal cortex after an injection of finasteride, further indicating allopregnanolone’s involvement with brain reward systems (Devoto, 2012). Based on this information, we hypothesized that administration of finasteride would result in increased stress induced amphetamine locomotor sensitization.

METHODS

Subjects: Thirty adult male Long-Evans rats (Dietham) were pair housed on a 12:12 light-dark cycle with lights on at 0700 hours. Rats were randomly assigned to one of three pretreatment conditions (saline, 10 mg/kg finasteride, or 25 mg/kg finasteride) followed by pseudo-random assignment to control or restraint stress conditions. Drugs: Finasteride (Stenekes, Inc., Newport, RI) was acutely administered by intraperitoneal (i.p) injection at a concentration of 130 mg/kg (21 mg/kg 5α-pregnanate) or in saline i.p in a concentration of 1.0 mg/ml. Pretreatment: Rats received i.p injections of either vehicle, 10 mg/kg finasteride, or 25 mg/kg finasteride both 48 hours and 24 hours before stress procedure. Acute stress: Twenty-four hours after the last pretreatment injection rats received control or restraint stress procedure (for instructions are administered on day 7). During 60 to 180 minutes after rats were in the stress group, placed in restraining tubes for 60 minutes while rats in the control group remained in their home cages. Experimental animals consisted of a total of 6 for both groups of 3 at each period: before stress, after one hour of stress control and 30 minutes after recovery. Amphetamine sensitivity: Twenty-four hours after acute stress, rats were transported to the testing room and allowed to habituate for 30 minutes. All rats received a saline injection (1 ml/kg) and were placed in an open field chamber for 30 minutes. After 30 minutes, rats were injected with 0.5 mg/kg of amphetamine and placed back in the chamber for 120 minutes. Distance traveled was collected using a S 38 photobeam tracking system (52x52x52 cm), and movements recorded for 120 minutes (20 cm). Supernatant was then filtered in a new eppendorf tube and samples were collected by 95 freezing. Allopregnanolone ELISA: ELISA kit was supplied by U.S. Life Science, Inc. 10-well strip plates were pre-coated with allopregnanolone antibody. Two wells were prepared for standard points, one well for blank. All samples, including the tissue were run in duplicate, 20 μl of standard, blank, and samples were pipetted into appropriate wells. Detection Reagent A was added to each well prior to one hour incubation. Following incubation, wells were aspirated aspirated and replaced. Detection Reagent B was added following 30 minutes incubation. Aspiration/wash process was repeated. Substrate solution was added to the wells followed by 15-minute incubation. Stop solution was added to all wells and plate was read through microplate reader at 450 nm to analyze absorbance. Corticosterone ELISA: Plasma samples were collected at baseline, during stress, and after stress for a total of three samples per animal, each only one of 20 μl of all trials provided sufficient plasma on all three samples to qualify for the assay. ELISA kit was supplied by Tec Bioscience, LLC. 96-well strip plates were precut with corticosterone antibody, 300 μl of albu buffer (12) were prepared with each well, 300 μl of standards and samples were pipetted into their respective wells. Additional 30 μl of assay buffer were pipetted into NIB wells. Blue corticosterone was pipetted into all wells, except blank, T, and NIB wells. The plate was incubated for 2 hours and then read at 405 nm to determine the average corticosterone concentration of each sample. Streptavidin–HRP conjugate was added to all wells, followed by one hour incubation. Stop solution was added to all wells and plate was read through microplate reader at 450 nm to analyze absorbance.

RESULTS

Allopregnanolone in Hippocampus

Conclusions

• One hour of acute restraint stress decreased overall locomotor activity in an open field.
• Finasteride did not lower allopregnanolone levels, regardless of dose.
• Pritchard et al. (unpublished) found similar results using a chronic stressor.
• Following 5 days of restraint stress rats exhibited locomotor sensitization in response to an acute injection of 1.0 mg/kg amphetamine. However, no effect of finasteride on amphetamine sensitivity was observed after this chronic stressor.
• Stereotypy was rated and effect of stress, not dose was found. Thus, rats that were stressed all demonstrated higher levels of stereotypy than control rats.
• Corticosterone levels did not elevate in response to stress.
• Limitations in our study may have been due to our timing of finasteride pretreatment, stressor type, or duration of stress. In addition, more thorough lipid extraction will be taken into consideration for future research.

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